part of the chamber around the tube shows strong coronas on exhaustion while the other half (toward the brass cap) is blank. Something, consisting of very slow-moving particles, gradually diffuses radially out of the aluminum tube. Of course it is difficult to deny with assurance that merest traces of emanation decaying within the aluminum tube may not possibly account for the activity; but what is remarkable in any case is the existence side by side of a region with nucleation and a region without it, in the absence of anything like a partition. The fog chamber itself must at all times be scrupulously free from infection such as an emanation would produce, and anything of this kind is at once detected.

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A New Method of Enumerating Bacteria in Air

The development of accurate bacteriological methods for the examination of air has not attracted wide attention in recent years; and this branch of bacteriology is far behind the related subject of water bacteriology in its technique and interpretation.

Bacteriological examinations of air have been carried out by most observers in one of two ways, without much attempt at critical control. The most primitive method consists in the simple exposure of plates of nutrient gelatin or agar for a more or less indefinite period. The colonies developing, correspond in a rough way to the bacterial flora of the air above. The method, however, can not be considered a quantitative one, since the number of bacteria which fall on the plate is not related to any particular volume of air and must vary with all sorts of environmental conditions. Nevertheless, this method is still used in many investigations in which quantitative results would be valuable; as in the important work of Major Horrocks on the presence of bacteria derived from sewage in ventilating pipes, drains, inspection chambers and sewers.[1]

[1] *Proceedings of the Royal Society*, Series B, Vol. 79, No. 531, p. 255.

The other method in common use is a modification of the sand-filter method of Pasteur and Petri. It involves the filtration through asbestos, sand, sugar, etc., of a measured volume of air; the washing of the filtering material with sterile water; and the plating of aliquot portions of the wash water in the usual way. Pasteur used asbestos for his filtering material; Sedgwick and Tucker recommended finely powdered sugar; and Petri and most recent observers have used sand. Petri pointed out that the sand should be of such fineness as to pass a .5-mm. mesh. In a recent important study of the air of the New York Subway Soper used both the plate method and the sand-filter method. The sand grains used were "about half a millimeter in diameter" and the sand layer 5 cm. deep.[2] In discussing these methods, in another paper, this author said, "as is well known, there is no precise way to determine the numbers of bacteria in air."[3]

I have been engaged for about a year in a study of bacteria in sewer air; and relied at first upon the sand-filter method. The remarkable results, reported by Major Horrocks in the paper to which reference has been made, led me to revise the detail of my technique with considerable care. In the course of the investigation a modified method of air examination was developed which is here reported in the hope that it may be of assistance to others at work on similar lines.

My aim was to combine the quantitative results of sand filtration with the directness and simplicity of the plate method. Hesse did this after a fashion by slowly aspirating air through a long roll-tube the walls of which were covered with melted gelatin. There was, however, a possibility in such an apparatus that bacteria might be drawn through, without settling out on the walls. My method is really a modification of Hesse's with an increase in the size of the culture vessel relative to the sample of air. I use two liter-and-a-half bottles arranged as shown in Fig. 1. On the

[2] *Technology Quarterly*, XX., 58.

[3] *Journal of Infectious Diseases*, Supplement No. 3, 1907, p. 82.

bottom of each is a layer of nutrient gelatin; and the tubing is adjusted so that a measured volume of air may be drawn through the two bottles in succession, by the action of a water-suction bottle, shown inverted on the right of the figure. In practise I place any desired amount of water, generally one liter, in the suction bottle and by slowly inverting it draw a corresponding volume of air from the bottom of the second culture bottle. The same volume of air passes from the bottom of the first bottle into the top of the second and from the outer air into the top of the first bottle. A known amount of air is thus drawn into the first bottle and the bacteria present settle out and form colonies on the gelatin. The volume of air examined being less than the capacity of either bottle, most of the bacteria remain in the first. A few, which are carried down by direct short currents, are caught in the second bottle. The results of a few examinations made by this method are shown in the table below.

The number of bacteria reaching the second bottle is evidently small, in most cases less than 10 per cent., and the number lost by being drawn through the second bottle must be negligible. With the exception of the possibility that bacteria may settle on the walls of the bottles, the method should give a complete account of all bacteria present which will grow under ordinary conditions of cultivation.

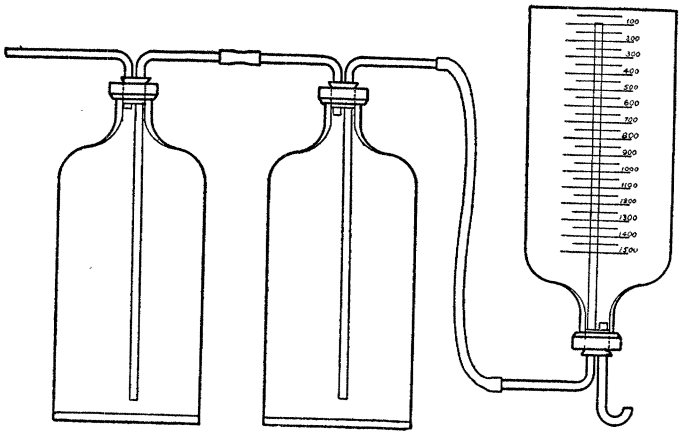

FIG. 1.

AIR EXAMINATION BY CULTURE BOTTLE METHOD

Air	Sample, c.c.	Colonies, First Bottle	Colonies, Second Bottle	Bacteria per Liter
Normal street air.	1,500	4	0	3
Air above foaming soapy emulsion of *B. prodigiosus.*	1,000	1	0	1
	1,000	1	1	2
	1,000	1	0	1
	1,000	3	0	3
	1,000	1	0	1
Air sprayed with suspension of *B. coli.*	100	73	1	740
	100	984	91	10,750
	100	394	69	4,630
Air sprayed with suspension of *B. prodigiosus.*	100	492	3	4,950
	100	320	34	3,540
	100	1,188	120	13,080

The culture-bottle method was devised primarily as a check on the sand filter method; and two types of sand filters were used for comparison. The first was the classic Sedgwick-Tucker apparatus, which consists of a glass tube 15 cm. long and 4 cm. in diameter, opening at one end into a smaller tube 10 cm. long and .5 cm. in diameter. A layer of 5 cm. of sand was supported in the small tube by wire gauze. A measured amount of air was drawn through, entering the larger tube and passing out through the sand. The sand, with the bacteria filtered out, was shaken down into the large tube, melted gelatin was added, and

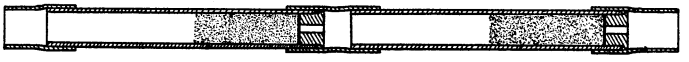

FIG. 2.

by rolling the tube on ice the gelatin with the sand and bacteria was cooled on its inner surface. The sand used in this filter was between .5 mm. and 1 mm. in diameter.

The other type of filter tested consisted of two short tubes, 1.5 cm. in diameter, arranged in tandem, each containing 2.5 cm. of fine sand, between .1 mm. and .3 mm. in diameter. The sand in each tube was supported by bolting cloth on a perforated rubber stopper and the tubes were connected by rubber tubing. The apparatus is shown in Fig. 2. After drawing air through this filter, the sand from each tube was shaken out into ten cubic centimeters of sterile water and, after thorough agitation, aliquot portions of the water were plated. This method is essentially the one used by Soper and by most recent observers.

Each of these filter methods is open to possibilities of error. Bacteria may be drawn completely through the filtering layer in either case; and in the second method there is danger that bacteria filtered out may not be separated from the sand or bolting cloth. My object was to find out the magnitude of these errors by direct comparison with the culture-bottle method. For this purpose a number of examinations were made, of normal air, and of air artificially infected with bacteria by spraying with emulsified cultures. With the filtration method samples of 750 c.c. to 1,500 c.c. were slowly drawn through the sand, the filtration occupying from two to three minutes. With the culture bottles, samples of 100 c.c. were generally used and the air was drawn in more rapidly. The general results obtained may be shown best by quoting a few typical experiments in detail.

Experiment III.—Examinations of air of a city street on a winter day. Four successive samples taken at intervals of fifteen minutes showed: (1) 3 bacteria per liter, by culture-bottle method; (2) 17 bacteria per liter by filtration method (fine sand); (3) 23 bacteria per liter by filtration method (fine sand); (4) 94 bacteria per liter by filtration method (fine sand). Apparently the number of bacteria in the air was increasing during this experiment; but the results by the two methods are concordant.

Experiment IV.—A suspension of a culture of *B. coli* was sprayed into a box and five samples taken at intervals of about ten minutes. The results were as follows: (1) 2,640 per liter by filtration method (fine sand); (2) 100 by filtration method (fine sand); (3) 740 by culture-bottle method; (4) 40 by culture-bottle method; (5) 0 by sand-filter method (fine sand). Evidently the bacteria were settling out rapidly. With the exception of the low sand-filter count in No. 2 the results of the two methods check fairly well.

Experiment V.—*B. coli* was sprayed into a box four times, at intervals of about ten minutes, a sample of the air being examined after each spraying. The results were as follows: (1) 175 bacteria per liter, by sand filtration (coarse sand); (2) 4,300 per liter by sand filtration (fine sand); (3) 4,000 per liter by sand filtration (fine sand); (4) 10,750 per liter by culture-bottle method. Very probably the repeated spraying more than balanced the settling out and the number of bacteria in the air of the box actually increased. The first result with the coarse sand seems low, however.

Experiment VI.—*B. prodigiosus* was sprayed into a box three times. The first two samples were examined after the first spraying, the third and fourth samples after the second and third sprayings, respectively. Results: (1) 15,000 bacteria per liter, by sand filtration (fine sand); (2) 14,000 per liter by culture bottle method; (3) 5,300 per liter by sand filtration (coarse sand); (4) 14,000 per liter by sand filtration (fine sand). Again the filtration method checked with the culture bottle method when fine sand was used, but gave low results with the coarse sand.

These experiments, and others of the same sort, seemed to indicate that sand filtration gives reasonably accurate results if the sand used be as fine as .3 mm. The crucial test of this point, however, must be made by drawing a given sample of air through sand filters and a culture bottle, so arranged in tandem that the bacteria which pass the sand shall be collected in the bottle. The table below shows a series of such experiments and makes it clear that the efficiency of the filtration method depends upon the size of sand grain employed.

RELATIVE NUMBER OF BACTERIA PASSING THROUGH SAND FILTERS; AND RETAINED IN THEM

Air Examined	Bacteria per Liter Retained in Filter		Bacteria per Liter Passing Filter
	Two 2.5 cm. Layers of .1–.3 mm. Sand	One 5 cm. Layer of .5–1 mm. Sand	
Suspension, *B. prodigiosus.*	100		2
Street air.	94		1
Suspension, *B. coli.*	2,640		12
" "		175	304
" "	4,000		37
Suspension, *B. prodigiosus.*		1,700	3,500
Suspension, *B. prodigiosus.*	14,000		2,400
Suspension, *B. coli.*	40		12
" "	90		15
" "		165	105

In seven tests with tandem sand filters, each containing 2.5 cm. of sand, with grains between .1 and .3 in diameter, the bacteria passing the sand were—once 30 per cent. of the number retained by the sand, twice 17 per cent., once 2 per cent. and three times 1 per cent. or less. On the other hand, in three tests with the Sedgwick-Tucker apparatus holding a single layer of sand, 2 cm. deep with grains between .5 mm. and 1.0 mm., nearly half the bacteria present passed the sand in one case and about two thirds escaped in the other two instances.

It seems clear that sand over .5 mm. in diameter is inadequate for filtering out bacteria. On the other hand, a sand finer than .3 mm. is generally efficient though not wholly reliable, since at times it allows a considerable proportion of bacteria to pass. This is not remarkable when the relative size of sand and bacteria is considered.

It is, of course, obvious that sand can not operate in the removal of bacteria by any process which can properly be called straining. In an editorial discussion of the removal of fine particles from water the *Engineering News* (LIX., 344) has described the phenomenon as "adhesion"; and the term deserves general acceptance in this connection. The size of the sand must affect the removal of fine particles in two ways. First, in a given depth, the number of surface contacts, which permit adhesion, must vary inversely with the size of the particles. Second, the velocity of flow, which tends to tear off adhering particles, must, under given conditions, increase with the size of the particles. Coarse sand might, therefore, be used with success by filtering through a deeper layer and by cutting down the rate of flow. It is simpler, however, to use sand sufficiently fine to regulate the rate of filtration automatically.

On the whole, the culture-bottle method seems to offer a more accurate procedure for bacterial examination of air than any yet available. The sand-filter method is fairly accurate as a rule, but occasionally gives low results. The filter method is more convenient than the culture bottle method for examinations outside the laboratory, since for the latter it is necessary to carry two 1,500 c.c. bottles for each examination. Aside from this difficulty of transportation, however, the technique of the culture-bottle method is to be preferred. Bottles are easier to prepare and to sterilize than sand filters and the actual examination is simplified by the omission of sand washing and subsequent plating.

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MASSACHUSETTS INSTITUTE OF TECHNOLOGY

SOCIETIES AND ACADEMIES

THE NEW YORK ACADEMY OF SCIENCES, SECTION OF ASTRONOMY, PHYSICS AND CHEMISTRY

A meeting of the Section of Astronomy, Physics and Chemistry was held at the Museum of Natural History on Monday, January 20, at 8:15 P.M.,